# MICROPROPAGATION OF *ROSA DAMASCENA* MILL.: THE EFFECTS OF GELLING AGENTS ON THE MULTIPLICATION STAGES AND ACCLIMATIZATION

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**Abstract.** The aim of our research was to investigate the way in which the agar types used for culture media preparation influence damask rose in vitro proliferation rates, as well as the quality of the shoots obtained and their ability for ex vitro rooting and acclimatization. For this purpose, seven types of gelling agents were tested: 6 g/L Daishin Agar, 4 g/L Gelcarin, 2 g/L Gelrite, 15 g/L Isubgol, 4 g/L Micro Agar, 6 g/L Phyto Agar and 4 g/L Plant Agar. The culture media consisted of Murashige & Skoog supplemented with 4 mg/L 6-benzyladenine. The highest multiplication rate, 5.1, was obtained on the culture media supplemented with 6g/L Daishin Agar and 4 g/L Plant Agar and the lowest one was 2.8, obtained on the media gelled with 4 g/L Gelcarin. Although the multiplication rate on the media gelled with Isubgol was lower (3.2), the shoots obtained in this experimental treatment got acclimatized with 90 % survival percentage and developed the best root system, regarding the number of roots/shoot and root length.

**Keywords**: Daishin Agar, Gelcarin, Gelrite, Isubgol, Micro Agar, Phyto Agar, Plant Agar

**Abbreviations**
BA- benzyladenine
BAP- 6-Benzylaminopurine
IAA-Indole-3-acetic acid
IBA-Indole-3-butyric acid
MS-Murashige and Skoog medium
NAA-Naphthalene Acetic Acid
$GA_3$-Gibberellic Acid
QL-Quoirin and Lepoivre medium
WPM- woody plant medium
TDZ- thidiazuron
Kin- kinetin

## INTRODUCTION

There are several studies that have been carried out on micropropagation of *Rosa damascena* Mill. (Alsemaan, 2013; Iliev et al., 2010; Jabbarzadeh and Khosh-Khui, 2005; Kornova and Michailova; 1994, Kornova et al., 2001; Mamaghani et al., 2010; Nikbakht et al., 2005), but the results upon the suitability of this method for multiplication of this species proved to be tardy and inefficient. Ginova et al. (2012) define *Rosa damascena* as recalcitrant species regarding its micropropagation and suggest that further studies are needed to be done to find the most suitable method for propagation with high multiplication rate and to ensure the genetic stability of the plants.

Previous studies that have been reported until now, were investigating all the micropropagation stages of this species and revealed that the existing micropropagation protocols applied, led to a very low propagation rate. Different treatments applied to the

culture medium were also extensively studied. Therefore, Jabbarzadeh and Khosh-Khui (2005) reported that the combination of BA at concentrations of 2.5–3 mg/L with a low rate of IBA was the most suitable treatment for in vitro multiplication of damask rose (3.75-4.00 shoots per single-node explant). Similarly, Alsemaan (2013) in one of his reports revealed that adding BA to the medium significantly increased the proliferation of the explants. In contrast, $GA_3$ did not have any significant effect on the proliferation, but the interaction of BA with $GA_3$ considerably increased the proliferation rate of the explants. The highest shoot proliferation was obtained in the presence of 2 and 2 mg/L of $GA_3$ and BA, respectively. Mamaghani et al. (2010) studied the effects of the culture medium and combination of various plant growth regulators on shoot proliferation of three elite Iranian *R. damascena* and announced, that the explants had higher shoot multiplication, shoot length and better green leaves on MS than WPM medium. The highest shoot proliferation (5.9) was obtained at a combination of 5 mg/L BAP and 0.1 mg/L TDZ. Maximum shoot length was observed in the medium with 0.5 mg/L IAA and 5 mg/L BAP and 0.01 mg/L TDZ. Shoot multiplication and shoot length were varied with genotypes and BAP as well as Kin concentration. M6 accession needed 2 mg/L BAP and 2mg/L Kin for maximum shoot multiplication and shoot length, G1 and G2 needed 2.5 mg/L BAP and 2.5 mg/L Kin.

Bhoomsiri and Masomboon (2003) in their research on damask rose observed that axillary shoot proliferation and root induction were achieved in the nodal segments on Murashige and Skoog (MS) medium and Quoirin and Lepoivre (QL) medium supplemented with benzyladenine (BA) or α-napthalene acetic acid (NAA) or in combination within 4 weeks. The best response in terms of frequency of shoot regeneration (100%) and maximum number of shoots (8.27 shoots per explant) were developed on QL medium containing a combination of 0.1 mg/L NAA and 4.0 mg/L BA. A proliferating shoot culture was established over repeated subculturing at 4-week intervals. Thus, 59.8 shoots were obtained in 8 weeks from a single nodal explant.

Besides, the weak in vitro shoot regeneration of damask rose, another difficult step in the micropropagation process, constitutes the rooting and acclimatization stage. Although, there were a few reports indicating high rooting percentage capacity. Thus, Bhoomsiri and Masomboon (2003) reported that rooting was obtained only on MS medium supplemented with 0.5 mg/L NAA (87.5 % of the explants exhibited root development with 2.71 roots per explant). The rooted plantlets (6-7 cm in length) were taken out from the culture flasks and washed to remove adhered agar and traces of medium. The plantlets were then transferred to soil (soil:meal of coconut fruit 1:1). Hardening of potted plants for 1 week under indoor conditions was found to be essential. The survival percentage of the plantlets was 90%, and plants were established under outdoor conditions.

Alsemaan (2013), in one of his research results mentions that the highest percentage of rooted explants (95%) was obtained in the presence of 3 mg/L IBA in the rooting medium, and the lowest (0%) was in the control treatment. The number and length of roots significantly increased in response to increasing IBA concentration in the medium up to 2 mg/L. The results showed that presence of IBA in the rooting medium is necessary for rooting of damask rose explants. The best results of rooting of the explants obtained by adding 2 mg/L IBA to the rooting medium.

Taking into consideration all the difficulties regarding the micropropagation of damask rose, the aim of our research was to investigate how different agar types used for culture media preparation can influence the in vitro multiplication rate of damask rose, as

well as the quality of the shoots developed and their ability for ex vitro rooting and acclimatization.

## MATERIALS AND METHODS

**Culture establishment**

In order to initiate the in vitro culture of damask rose, the 1.5-2 cm long explants were picked from the annual shoots of the mother plant of *Rosa damascena* Mill. cultivated as potted plant in the greenhouse. The single-nodal stem explants were washed with tap water first, then rinsed with sterile deionized water, followed by a 20 minutes disinfection with a 20% chlorine solution (ACE solution has 5% active chlorine bleach). The 0.5-11 cm long explants were cultured on a 10 ml Murashige and Skoog (1962) basal salts and vitamins supplemented with 30 g/L sucrose and gelled with Plant Agar in 320 ml glass jars. After 8 weeks of growth the first subculture was transferred to a solid MS medium supplemented with 5 mg/L 6-benzylaminopurine (BAP). The second subculture was transferred after another 8 weeks of growth to a fresh solid MS medium prepared with 4 mg/L BAP.

**The multiplication processes**

The explants used in the multiplication process were taken from the previously established 10-week-old in vitro culture of damask rose, cultured on MS medium supplemented with 4 mg/L 6-benzylaminopurine (BAP) and gelled with 4 g/L Plant Agar. Growth induction of axillary shoots and multiplication of shoots were assessed using nodal segments with two axillary buds excised from shoots. The inoculation of the 5 explants per jar with 5 repetitions was made at an angle thus approximately two-thirds or three-quarters of the explants' lengths had direct contact with the culture medium.

*Culture medium:* In this experiment 7 types of culture media were tested, prepared from the Murashige & Skoog 1962 (in powdered form from Duchefa Biochemie B.V.) basal salts supplemented with 30 g/L sucrose (Cristal from the trade market) and 4 mg/L 6-Benzyladenine (BA) provided by Duchefa Biochemie B.V. and gelled with the following agents:

V1 - Daishin Agar 6 g/L (Duchefa Biochemie B.V);
V2 - Gelcarin 4 g/L - (Duchefa Biochemie B.V);
V3 - Gelrite 2 g/L - (Duchefa Biochemie B.V);
V4 - Isubgol 15 g/L – (Natural Brand™ Colon Pure™ Unflavored, Code 350971);
V5 – Micro Agar 4 g/L - (Duchefa Biochemie B.V);
V6 – Phyto Agar 6 g/L - (Duchefa Biochemie B.V);
V7 – Plant Agar 4 g/L – (Duchefa Biochemie B.V).

All the components were added to the media before autoclaving. The pH of the culture media was adjusted to 5.8±0.1 with NaOH or HCl after the growth regulators were added. The culture media were distributed in glass containers (50 ml for each) and autoclaved for 20 minutes at 121 ºC.

*Culture containers:* Glass jars of 320 ml were used with filter on their lids to prevent the passage of micro-organisms and microbial spores. For this reason, the holes on the lids were stuffed with a small piece of heat resistant sponge (18 mm x 18mm).

*Incubation of the cultures:* The cultures were incubated in a growth chamber at 23±2 °C with a 16 h light/8 h dark photoperiod. The light intensity was 36 μmol $m^{-2}$ $s^{-1}$ from cool white fluorescent tubes.

**Rooting and gradual acclimatization to in vivo conditions of the in vitro plants**

Rooting and acclimatization of the plants were done simultaneously:

1. *Rooting and acclimatization in floating perlite without hormones.* In this case the newly developed individual shoots without roots taken from all the 7 types of media were introduced in floating perlite at equal distances between them according to the acclimatization method provided by Clapa et al. (2013) at the UASVM/HRS Cluj-Napoca. The cultures were partially covered (80 % of the area of the vessel opening) with plastic lids for 2 weeks to ensure protection and suitable air circulation.
2. *Rooting and acclimatization in floating perlite with growth regulators:* In this case 1 mg/L IBA was added to the water before adding the perlite and the rest of the procedure being similar to the previous one as mentioned above. Rooting and acclimatization of the shoots took place in the Laboratory of Micropropagation of UASVM.

**Statistical analysis**. All recorded data were subjected to analysis of variance (ANOVA) and then compared by Tukey's multiple range test ($P \leq 0.05$). The data shown are mean values ±SE.

## RESULTS AND DISCUSSIONS

**The influence of the gelling agent from the culture media upon *Rosa damascena* Mill. in vitro multiplication**

Regarding the seven gelling agents tested, the highest number of shoots/vessel, 5.08 ± 0.1138 respectively, were obtained in the treatment V1 – MS+4 mg/L BA+6 g/L Daishin Agar and the smallest number of shoots/vessel was obtained in the treatment V2 - MS+4 mg/L BA+ 4 g/L Gelcarin, 2.8±0.1028 respectively (Fig. 1 A).

Likewise, on the media gelled with 6g/L Daishin Agar the longest shoots were obtained, with an average length of 9.14±0.1930 cm, whereas on the medium gelled with 4 g/L Gelcarin the shortest shoots were obtained, with an average length of 5.4±0.2039 cm (Fig.1. B), which is agreement with the results reported by Alasemaan (2013), who obtained shoots of 9.2 cm maximum length on MS media supplemented with 2 mg/L BA and 2 mg/L $GA_3$.

The highest multiplication rate was 5.1, obtained on the media variants gelled with 6 g/L Daishin Agar and 4 g/L Plant Agar respectively. Similar results were reported by Mamaghani et al. (2010), who obtained the highest multiplication rate (5.9) at a combination of 0.1 $mgl^{-1}$ IBA, 5 $mgl^{-1}$ BAP and 0.1 $mgl^{-1}$ TDZ.

In this experiment, the lowest multiplication rate was 2.8, obtained on the media gelled with 4 g/L Gelcarin (Fig. 1. C). A similar multiplication rate was reported by Kornova et al. (2001) for *Rosa damascena* Mill who tested 11 different media on the base of MS with participation of auxins NAA and IAA (0.1 $mgl^{-1}$). The most appropriate medium for micropropagation found by Kornova et al. (2001) was the MS containing BAP 0.5 -1 $mgl^{-1}$ with or not 0.1 $mgl^{-1}$ IAA with optimal rate of multiplication 2.2 -2.6.

**Direct ex vitro rooting and acclimatization.**

*Ex vitro rooting and acclimatization in hormone-free floating perlite.*

Methods of direct ex vitro rooting concomitant with acclimatization have not been reported by other researchers; in all the studies in vitro rooting was used, followed by the ex vitro acclimatization of the shoots rooted in vitro (Alsemaan, 2013; Jabbarzadeh and Khosh-Khui, 2005; Kornova et al., 2001; Mirza et al., 2011).

The in vitro rooting percentages varied depending on cultivars, culture media or the hormones used. Alsemaan (2013) reports the highest in vitro rooting percentage in *Rosa*

*damascene* (95%) on MS media supplemented with 3 mg/L IBA, as compared to 0% on the same media without growth regulators.

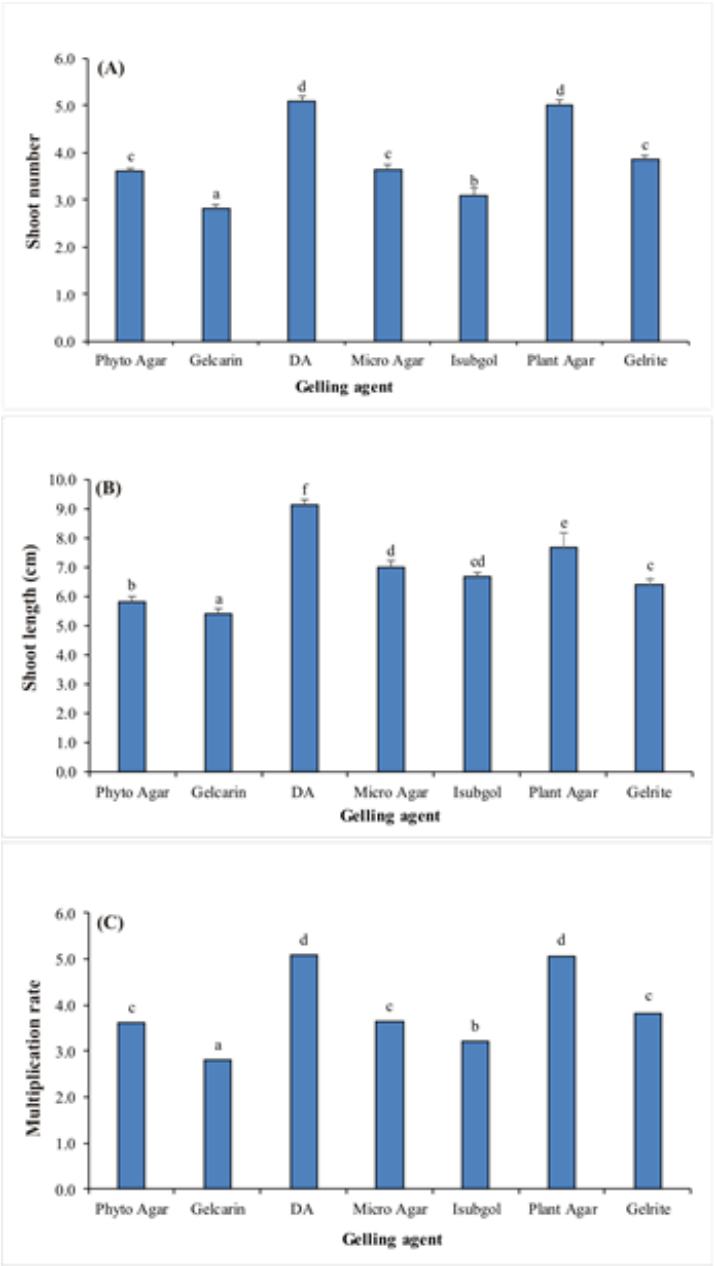


Fig.1. The influence of the gelling agent on shoot number (A), shoot length (B) and multiplication rate (C) of *Rosa damascena* Mill. The data were analyzed using one-way analysis of variance followed by Tukey's HSD test. The different lowercase letters above the bars indicate significant differences among the gelling agents' effects according to the Tuckey's HSD test (P=0.05).

Ex vitro rooting in floating perlite without growth regulators proved to be easy and effective in *Rosa damascena* Mill. The rooting percentage was 90% in the shoots resulted

from the multiplication media gelled with 15 g/L Isubgol and 50% in the shoots resulted from the multiplication media gelled with 6g/L Phyto Agar (Fig. 2 A) with very well-developed rootlet (Fig.2 C).

The shoots regenerated on MS with 4 mg/L BA gelled with 15g/L Isubgol presented the highest number of roots/shoot, 3.2 respectively, and the highest average root length of 9.07 cm after four weeks of culture in floating perlite (Fig. 2 B, C). In comparison, Bhoomsiri and Masomboon (2003) obtained 85% in vitro rooting percentage on MS supplemented with 0.5 mg/L NAA. The rooted shoots had an average number of 2.3 roots/shoot after four weeks of in vitro culture and the acclimatization percentage of the shoots rooted in vitro was 90%.

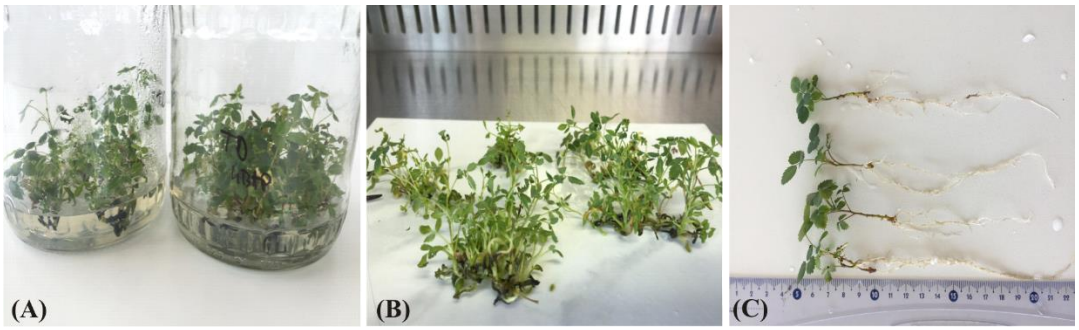


Fig. 2. Micropropagation stages of *Rosa Damascena* Mill: A-Establishment of aseptic culture; B-Multiplication of shoots; C) Ex vitro rooting and hardening of the plantlets in floating perlite without growth regulators.

The high rooting percentage of the shoots regenerated in the multiplication stage in the experimental treatment with MS media supplemented with 4mg/L BA and gelled with 15g/L Isubgol was due to shoot vigorousity.

The application of both ex vitro rooting and acclimatization methods reduces the costs of micropropagation in *Rosa damascena* Mill.

***2. Rooting and acclimatization in floating perlite with 1 mg/L IBA .***

In this case rooting percentage was zero, IBA showing no effect on rooting. Moreover, during the first week after transferring the shoots into the floating perlite all the shoots were affected by necrosis.

## CONCLUSIONS

According to our investigations, the gelling agents used for the culture media strongly influenced the in vitro proliferation rate in *Rosa damascena* Mill. therefore, we recommend further in-depth research on this issue. The one-step direct ex vitro rooting and hardening-off in floating perlite, without growth regulators can be a solution for the acclimatization of *Rosa damascena* Mill. axillary shoots obtained in vitro in the multiplication stage, with the condition that these should be well-developed and vigorous. This method reduces the effective costs of producing *Rosa damascena* Mill. planting material by micropropagation, as the in vitro rooting stage is eliminated.

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